Abstracts

Trace Metals in the Marine Environment

Cycling of Trace Metals in the Newport River Estuary.

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At the Atlantic Estuarine Fisheries Center, efforts are under way to determine the cycling of toxic metals in a single estuarine system, particularly as it relates to the flow of energy among dominant estuarine species and the physiological effects of metals, thermal additions and chlorination on these organisms. We have studied the distribution of manganese, iron, zinc, and to a lesser extent Cu, among sediments, water, and biota in the Newport River estuary. These distributional data have been coupled with the results of other ecological research conducted at the Center on biomass distribution and biological productivity and preliminary data on hydrology of the estuary. Through these analyses, we have estimated both the effect of biological and physical processes on the flux of metals through the estuary and the flux of metals in certain food webs within the estuary. The results of these efforts are discussed in terms of the effects of environmental variables on accumulation of metals in marine organisms.

Zinc Turnover in the Mosquitofish: Measurement Techniques.

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Radioisotope techniques used to measure rate constants and sizes of the mathematical components of Zn turnover in various organisms have often given erroneous component parameters because the ^{65}Zn was not distributed uniformly throughout the entire organism. A technique was devised to overcome this deficiency and to compare the component rate constants and sizes obtained from experiments utilizing loss of tracer with those utilizing uptake of the same tracer.

Forty newly-born mosquitofish, *Gambusia affinis*, were reared to adulthood in an environment uniformly labelled with ^{65}Zn. Forty others were reared in a similar nonlabeled environment. The fish were then switched from the labeled to the nonlabeled environment and vice versa, and either the accumulation or retention of ^{65}Zn in each fish periodically determined. However, one-third of the fish were placed individually into 10 liter plastic containers in their new environment, one-third individually into 57 liter cages inside 2000-liter fiberglass tanks, and one-third in a group into 2000-liter fiber glass tanks similar to the ones in which they were reared. The turnover rate constants and component sizes of Zn were determined from the ^{65}Zn accumulation and retention curves. These parameters as determined from retention were different from those determined from accumulation in every case except that in which the fish were placed together and allowed to swin freely in the 2000-liter tanks. These experiments indicated that experiments for investigating the turnover of stable metals should always be conducted in an environment as similar as possible to the natural environment of the organism.

Factors Affecting Mercury Concentrations in Recent and Old Bathyl-Demersal Fish.

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Total mercury concentration in the axial muscle, liver, brain, and gill tissues of three species (*Antimora rostrata*, *Aldrovandia* sp. and *Chalinura* sp.) of bottom-dwelling fish from 2800 m differed significantly. The mercury content in the axial muscle within only the recent and the old *Antimora rostrata* increased significantly with size. The concentration of mercury in the livers of *Aldrovandia sp.* differed by an order of magnitude from muscle concentrations. Gill tissue concentration remained the same in all three species. Brain tissue concentration showed no pattern. Possible factors affecting the introduction of mercury and the variation within the group were investigated.

Heavy Metal Concentrations in Museum Fish Specimens: Effects of Preservatives and Time.

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Specimens of myctophid fishes preserved for one month in formalin, ethyl alcohol, and isopropyl alcohol had higher concentrations of cadmium, copper, zinc, and sometimes lead and lower concentrations of mercury and sometimes lead than did unpreserved frozen specimens. Properties of the preservatives and species differences in fish tissues both influence these metal concentrations. Maximum concentrations of some metals in preserved specimens appear to be attained within a month, while concentrations of others may continue to increase for years. Metal tags or other materials in the preservative may cause higher maximum concentrations than the preservative alone. Comparisons of concentrations of metals between museum specimens and unpreserved (frozen) specimens must be considered unreliable until the changes resulting from preservation are understood.

Behavior: An Index for Establishing Sublethal Toxicity Levels for Heavy Metals.

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This study examined the sublethal effect of mercuric chloride on the conditional avoidance response of female grass shrimp, *Palaemonetes pugio*. The LD_{50} for 120 hr exposure was 200 ppb; the maximum sublethal dosage (based on 120 hr exposure) employed in these experiments was 50 ppb. Animals were collected at low tide near Beaufort, N.C. and were reared in natural seawater at controlled temperature, salinity and photoperiod. The Lafayette 88000 Student Avoidance Apparatus consisting of an avoidance conditioning tank, programmer timer, and shock console was used to condition shrimp to avoid a shock by leaving the illuminated side at the onset of light. Three treatment groups, each with five replicates were examined: (1) shrimp trained until a stable score was obtained and then dosed (TTD); (2) those trained while dosed throughout the experiment (DAT); (3) those trained and never dosed served as controls (CT). Each replicate in a treatment group contained 5 shrimp (or 25 animals/treatment). A stable score was one which varied no more than 3% for 5 consecutive days. To obtain a correct score those shrimp swimming in the half of the tank where light appeared were required to move past the midline of the tank to the dark, unelectrified side within the 5-sec light phase. Scores expressed as percentages of the total possible correct responses were obtained by counting the number of shrimp escaping the lighted side within the first 5 sec of light and divided by the number of shrimp present on that side at the onset of light. All shrimp were presented with 25 training trials/day for 29 days. During the first 30 days the CT and TTD had similar avoidance responses, however both were significantly different from the DAT. On the 21st day the TTD was dosed and 48 hr later their avoidance responses dropped to the level of the DAT. On the last day of testing, the CT was significantly different from both the TTD and DAT groups, but the DAT was not significantly different from the TTD. There was more mortality in the TTD and DAT groups than in the CT, but differences were not significant. Although 50 ppb was sublethal during early summer, in the early fall it was found that 25 ppb was a lethal dosage. Those environmental factors which vary toxicity with season will be studied in the future.

Trace Metal Uptake and Toxicity to Shellfish.

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The relationship between uptake and toxicity of trace metals to shellfish is of interest because these organisms are an important food source and human trace metal poisonings have occurred through their consumption. An understanding of how cells of marine invertebrate tissues handle trace metals in comparison with mammalian cells is also of scientific value.

In the current study, randomly selected specimens of the claim, *Mercenaria mercinaria*, and the oyster, *Crassostrea virginica*, were placed in sea water solutions (26°C and 35% salinity) containing 0, 0.1, 1, or 10 ppm Hg^{2+} or Cd^{2+}. The experiment was terminated for each element after half of the animals in the 10 ppm dose groups exhibited gaping valves. Cross sections of the animals were studied by light microscopy and portions of the mantles by electron microscopy. Heavy mortality occurred in oysters of the high dose groups after about 3 days of Cd^{2+} or Hg^{2+} exposure. This effect was not seen in high dose claims until 5 days with Hg^{2+} and 7 days with Cd^{2+}. The most readily detectable histologic changes characterized by characterized by clumping nuclear material and cytoplasmic rarification were observed in columnar epithelial cells lining the gut of each species. Electron microscopy of mantle epithelial cells disclosed a dose-

dependent increase in electron dense cytosomes within these cells in Hg^{2+}-treated claims. The cytosomes were not observed in Cd^{2+}-treated animals or in oysters exposed to Hg^{2+}. Energy-dispersive x-ray microanalysis disclosed the presence of high iron concentrations in reaction to mercury within these cytosomes.

Trace Metals in the Agricultural Ecosystem

The Movement Through Soil and the Uptake, Accumulation, and Mineral Metabolic Imbalances Produced by Cadmium in Field-Grown Corn, Soybean, and Peanut.

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Currently there is an increasing interest in utilizing agricultural soils as receiver systems for municipal sewage sludges. Heavy metals, including Cd, are frequently present in substantial quantities in such materials and a number of other current agricultural practices add Cd to our soils. Because of our concern about the hazards to plants, animals, and humans of increased levels of Cd, experiments were done to determine the rate and extent of Cd movement in a sandy loam soil and its relationship to Cd uptake, accumulation and physiological effects on field-grown corn, soybean, and peanut. In May 1973, an aqueous solution of $CdCl_2$ was sprayed onto the surface of replicated plots in sufficient quantities to theoretically bring the top 15 cm soil layer to final Cd concentration of 0, 3, 11, and 20 ppm, respectively. In order to determine the extent of interactions between Cu, Zn, and Cd under field conditions, another series of plots received either sprays of $ZnSO_4$ and $CuSO_4$ for final concentrations of 20 ppm of Cu and Zn, or sprays of Cu, Zn, and Cd, all at 20 ppm. Soil and plant samples were taken periodically from June to October. After solubilization of the soil cations in a diethylenetriamineacetic acid (DTPA) extraction solution, Cd, Cu, and Zn were determined by atomic absorption spectrophotometry. Plant tissues were oven dried at 100°C, ground with a Wiley mill to pass a 20 mesh screen, wet digested with nitric and perchloric acids, and analyzed for their Cd, Cu, Zn, Fe, Mn, Ca, and Mg contents by atomic absorption spectrometry. In subsequent experiments designed to study the interaction between Cu, Zn, and Cd and the effect such interactions have upon Cd tolerance, it was found that while 1:1 molar ratios of Cu:Cd and 1:1:1 ratios of Cu:Zn:Cd were effective in lowering the amount of Cd incorporated into both tolerant and nontolerant isolates, the 1:1 ratios of Zn:Cd had little effect. The threshold concentration for the protective effect of Cu was found to be at 100μM for the nontolerant and at 1000μM for the tolerant isolate. During late phases of growth (2–3 weeks), the protective effect of Cu began to disappear. None of the Cu or Zn treatments used was effective in overcoming the growth-inhibitory effects of Cd.

Factors Affecting Cadmium Tolerance and Mineral Metabolism in *Sclerotinia homeocarpa*.

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Golf course operators have used cadmium containing fungicides frequently at rates as high as 215 lb Cd/acre/yr for the control of the dollar spot pathogen, *Sclerotinia homoeocarpa*. Increasing difficulties in control of the disease led Cole to study the comparative Cd tolerance of a large number of fungal isolates derived from Cd-treated bentgrass. He found isolates that were inhibited by 10 ppm Cd and isolates that grew well in the presence of 1000 ppm Cd. Our research is being done by use of isolates obtained from Cole and is designed to determine the physiological basis of the Cd tolerance in this organism. From among 40 isolates, a tolerant and a nontolerant isolate were selected for these investigations based upon their tolerance of Cd *in vitro*. In one series of experiments, the fungi were grown for varying lengths of time at 28°C in liquid media containing from 0 to 300 ppm Cd. After addition of acetone to kill the fungus, the mycelium was filter-washed, dried at 100°C, weighed, ashed in nitric and perchloric acids, and elemental analyses were performed by atomic absorption spectrophotometry. On a dry weight basis, the mycelium of the tolerant isolate was found to accumulate 128-fold less Zn, 67-fold less Cu, and 37-fold less Cd than the nontolerant isolate. The ([Cu] + [Zn])/[Cd] ratios of the nontolerant isolate remain higher than those of the tolerant isolate. It appears that one factor of the tolerant isolate's Cd tolerance is its capacity to maintain nearly normal Cu levels while the nontolerant isolate exhibits as much as a 100-fold increase. The data revealed that Cd movement in the soil was not affected differentially by the three crop species but did show significant cadmium concentration–rate of movement interactions. In general, the more Cd applied, the faster and the further the Cd moved in the soil. At the first sampling time, the extractable Cd values for all crops at the 0, 3, 11, 20 ppm treatments at the 0–5 cm sampling depth were 0.62, 2.91, 13.60, and 22.46 ppm Cd. At the 5.1–10.0 cm depth,

the values were 0.08, 0.23, 0.73, and 4.32 ppm Cd, respectively. At the 10.1–15.0 cm depth, values were 0.05, 0.09, 0.16, and 0.63 ppm Cd, respectively. While some Cd was detected at the 15.1–20 and 20.1–25 cm increments, a concentration of 0.50 ppm Cd was never exceeded. The presence of added Zn and Cu had no effect on the soil distribution of Cd. While there was some downward movement of the Cd with time, most of it remained in the surface soil horizons. Thus, it is unlikely that significant amounts of Cd when applied to sandy loam at concentrations less than 20 ppm will contribute much Cd to the subsoil and ground water via leaching mechanisms. Elemental analyses of leaf tissue collected during the summer and of mature seed collected at harvest time revealed that Cd was taken up and translocated to all plant parts. The amount present was a function of time, concentration of Cd applied and species of plant. Seed Cd concentrations at the 0, 3, 11, and 20 ppm Cd treatments were 0.12, 0.66, 0.89, and 1.14 ppm for corn, 0.28, 1.33, 1.11, and 1.05 ppm for soybean, and 0.45, 1.10, 4.30, and 8.28 ppm for peanut. In comparison with the treatments where Cd only was applied, the simultaneous addition of Cu, Zn and Cd resulted in a 20–40% decrease in the amount of Cd incorporated into the seed.

Aerial Pollution and Copper Tolerance in *Agrostis stolonifera.*

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A metal refining industry at Prescott in SW Lancashire, England, has caused considerable aerial pollution on the surrounding vegetation. The soil and plants were analyzed. It was clear that the major heavy metal content in the soil is copper. Marked copper tolerance of different degrees in different individuals was found in the contaminated populations of *Agrostis stolonifera.* The physiological characters copper uptake, root respiration, and malate dehydrogenase (MDH) activity were studied to investigate the differences in the response to copper toxicity between tolerant and nontolerant clones. It was found that the roots took up far more copper than leaves. The roots of the tolerant clone took up more copper than the nontolerant clone in the equivalent copper concentrations. The roots of the tolerant and nontolerant clones were treated in two ways before they were used for the measurement of respiratory rates and MDH activities: (1) the fresh roots were produced by growing the tillers in calcium nitrate solution (0.5 g/l.); (2) the copper-pretreated roots were treated by growing the rooted tiller in copper solutions for certain times. The respiratory rates of the excised fresh roots, to which the copper was added in the incubation medium in Warburg flasks, were not different between the tolerant and nontolerant clones and between the different concentrations of copper in the incubation medium. However, the roots pretreated with copper showed that the respiratory rates were greater in the tolerant clone, and were reduced less than in the nontolerant clone with increasing copper concentrations. The MDH activities present in extracts of fresh untreated roots were found to be the same for tolerant and nontolerant clones. When copper was added to the enzyme reaction mixture it was only found to inhibit activity of the enzyme at a concentration about 180μM and did not discriminate between the two clones. The MDH activity detected in enzyme extracts prepared from roots pretreated with copper for 8 days showed remarkable reduction when the enzyme activity was expressed as a fraction of fresh weight, and the MDH activity of the nontolerant clone was reduced more seriously than the tolerant clone. But when the MDH activity was expressed in relation to protein content in the enzyme extracts, no difference was found between the two clones. The copper content in the soil of the copper refinery area is at a very high level, the average water-soluble copper content occurring up to 20 ppm, and this is much higher than the copper concentration (0.63 ppm) which seriously inhibits root growth of both copper tolerant and nontolerant clones in calcium nitrate solution. Thus it is pertinent to look at possible external mechanisms reducing toxicity, since this evidence suggests that in certain circumstances the toxicity of the metal ions is prevented. The tillers of both tolerant and nontolerant clones were grown in nutrient culture solution. The macroelements P, Ca, K, Mg, and EDTA were omitted from the culture solutions and copper was added at 1, 2, 5, and 10μM final concentrations. No inhibition of root elongation was detected when the copper tolerant clone was cultured in any of the four copper concentrations and lacking in turn just one of the four macronutrient elements or EDTA. However, the root elongation of the nontolerant clone was seriously inhibited by 2μM copper when EDTA was absent from the nutrient solution, and by 5μM when phosphate was absent from the nutrient solution and was seriously inhibited by 10μM copper when K or Mg was absent. When calcium was absent in the nutrient solution, the root elongation of the tolerant clone in the culture solution was inhibited even without copper being added, and the root elongation was seriously inhibited by 10μM copper. This different response to calcium between the two clones in the absence of copper may be related to the fact that the nontolerant clone came from a high calcium habitat (sand dune), whereas the Prescot soil is probably lower in this element. The studies of the effect of macronutrient elements demonstrate that the macronutrient elements could be responsible for the reduction of the copper toxicity in the soil in the copper refinery area. The gardeners use fertilizer for the

lawns at Prescott in the normal manner. This would reduce the copper toxicity in the soil considerably.

Studies on the Absorption of Zinc, Mercury and Cadmium.

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Studies have been conducted to examine the distribution of zinc, mercury, and cadmium in the cytosol of mucosal cells of chicks fed these elements. The control chicks were fed a corn–soybean meal diet without added zinc, cadmium, or mercury, while the treated animals received this diet supplemented to contain either 200 ppm zinc or cadmium or 500 ppm mercury. The elements were added to the diet in inorganic forms. Supplementation with zinc or cadmium led to the appearance of a protein of approximately 13,000 molecular weight which bound cadmium. Supplementation with zinc, but not cadmium, led to the appearance of a zinc-binding protein of the same size. Supplementation with both zinc and cadmium also failed to demonstrate the appearance of the zinc-binding protein. These results suggest that cadmium induction of this protein results in cadmium being so tightly bound that zinc cannot exchange with it. Supplementation of the diet with mercury did not result in the appearance of a 13,000 molecular weight protein which bound mercury, although mercury would bind to the cadmium-induced protein.

Biological Interactions of Sulfate and Molybdate.

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We have previously proposed that sulfate and molybdate interact in the following two systems in ruminants: (a) the sulfate-reducing system present in the rumen, and (b) the membrane transport system. *In vitro* studies with rumen microorganisms from sheep fed sulfate showed that molybdate inhibits system (a) which reduces sulfate to sulfide. *In vivo* studies by Mills with sheep fed natural diets indicated, however, that sulfide levels increased rather than decreased in the rumen when supplemental molybdate and sulfate were fed. In order to determine the effect of dietary molybdate on sulfide production, twelve fistulated sheep were fed purified nonprotein diets; and sulfur was supplied to six of the animals as sodium sulfate and to the other six as DL-methionine. Three sheep from each of these two treatments were fed 50 ppm molybdenum as sodium molybdate. The rumen microorganisms were assayed for sulfide production, and comparisons were made between molybdenum fed and non-molybdenum fed animals. These studies showed that the concentration of Mo (4.5 ppm), which caused a 31% inhibition of sulfate reduction *in vitro* in the sulfate animals not fed Mo, caused only 36% inhibition in the Mo-fed sulfate animals. In the animals fed the methionine diet, the concentration of Mo (4.5 ppm), which caused a 47% inhibition of sulfide production *in vitro*, caused an 83% increase in sulfide production when the Mo was fed. These results confirm that molybdate interacts with sulfate antagonistically in the sulfate-reducing system present in the rumen of sheep and indicates that the increased sulfide production observed by other investigators when ruminants were fed protein diets plus molybdenum may be due to an enhanced production of sulfide from sulfur amino acids. Studies of the proposed interaction of sulfate and molybdate in the membrane transport system (b) were conducted with both ruminants and nonruminants by the *in vivo* intestinal loop technique. Isolated duodenal loops were injected with either ^{99}Mo-labeled Na_2MoO_4 (0.1 μ moles) alone or with Na_2SO_4 (40 μ moles). In these experiments, sulfate inhibited the absorption of $^{99}MoO^{-2}_4$ in rats (20%), chicks (30%), and sheep (75%).

Analysis, Biochemistry, and Toxicology of Trace Metals

Evaluation of Arsenic in Urine and Water by Using Flameless Atomic Absorption Spectrometry and Electrodeless Discharge Lamp.

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A method is described for the low-level evaluation of arsenic in water and urine by flameless atomic absorption spectrometry and an arsenic EDL lamp, but which eschews the intermediate generation of arsine and problems attending this procedure. Water and urine samples are first warmed with hydrochloric acid and potassium iodide to effect generation of the trivalent arsenic iodide(s). Extraction of these media is then carried out by using chloroform presaturated with the hydrochloric acid normality used in analysis. Back-extraction of the chloroform extracts with deionized water is then carried out, which liberates arsenic into the aqueous layer via hydrolysis. Aliquots of the water layer are introduced into the Massmann furnace accessory of a Perkin-Elmer Model 403 spectrometer, where they are subjected to drying, ashing

and atomization steps. The lower detection limit is 20 ppb arsenic, while nonlinear response appears above 5 ppm arsenic. At levels of 50 ppb arsenic, the relative standard deviation is 2.6.

Preliminary Gas-Liquid Chromatographic Studies of Inorganic and Organic Arsenicals.

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Inorganic, monomethyl-, and dimethylarsenic (III), as the corresponding diethyldithiocarbamates, have been prepared and studied by gas-liquid chromatography, by using an instrument equipped with an electron-capture detector (^3H foil). All three compounds have been found to chromatograph without difficulty when using a glass column, 18 in. long and packed with silanized 5% OV-17 on Anakrom A S., 80/90 mesh. Periodic resilanizing is necessary to retain elution properties for the arsenicals. Atomic absorption spectral analysis of the materials eluted from the column which give rise to the peaks confirm the presence of arsenic. Tris(diethyldithiocarbamato)-arsenic (III) is chromatographed at 160°C and a lower detection limit of 73 ng/ml benzene; the monomethyl derivative at 110°C with a lower detection limit of 40 ng/ml benzene, and the dimethyl compound 70°C with 15 ng As/ml benzene as the lower limit. Preliminary results involving application of GLC assay to the above arsenic compounds contained in various media will be presented.

Characterization of Low Molecular Weight Copper-Binding Protein From Rat Liver.

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Intraperitoneal injection of $CuCl_2$ into rats leads to the formation of a cytoplasmic copper-binding protein (CuBP). The protein has been purified by Sephadex gel filtration and acetone fractionation. CuBP exhibits an apparent molecular weight of about 8000 daltons and is distinguishable from Cd-thionein by electrophoretic mobility and amino acid composition. Ultraviolet absorption spectroscopy reveals an intense absorption band in the region of 250 nm, which is absent in the apoprotein. As purified, the protein has a Cu content of 2.5%, Cu being the only metal detected. EPR studies reveal that the Cu in CuBP is in a diamagnetic state, but can be converted to the paramagnetic state by incubation with PCMBS or ferricyanide. CuBPs isolated from rat kidney, chicken liver, rabbit liver, plants, and yeast show similar properties.

Copper-Induced Synthesis of Copper-Binding Protein in Rat Liver and Yeast.

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A low molecular weight copper-binding protein (CuBP) has been purified from the livers of rats injected with copper and from yeast grown in presence of copper. In rats, injection of copper stimulated the incorporation of [4, 5-^3H] lysine into liver CuBP with the concomitant increase in the hepatic copper levels. Pretreatment of the animals with cycloheximide or actinomycin D prevented the incorporation of the label into the protein. The inhibitors, however, did not affect the hepatic uptake of copper. In animals treated with the antibiotics, the hepatic copper was bound nonspecifically to the soluble proteins. The stimulated incorporation of [5-^3H] orotic acid into rapidly labeled liver RNA brought about by copper injection was abolished when the animals were pretreated with actinomycin. The increased incorporation of [4, 5-^3H]-lysine into CuBP resulting from exposure of yeast to copper was abolished when the yeast cells were pretreated with cycloheximide. These results are interpreted as indicating that copper induces the synthesis of CuBP and that it exerts its control at the transcriptional level.

Distribution of Lead in Subcellular Fractions of Cerebrums of Guinea Pigs.

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Guinea pigs given large doses of lead orally had their cerebrums removed and separated into subcellular fractions which were measured for lead and protein. The subcellular fractions were obtained by using the method of Kurokawa, Sakamoto, and Kato; and consisted of the nuclei, mitochondria, myelin, nerve endings, microsomes, and soluble proteins. Two studies were performed. One measured the long-term effects of previous lead dosage, and the other measured sequential changes over 5 days of acute lead poisoning. In the first study, lead was found to be associated with nuclei and mitochondria slightly more than with the other subcellular fractions (which were very similar in lead content). In the second study, lead was found to increase steadily in the cerebrum in the early phases of acute poisoning, and then to increase massively just before the time of the onset of convulsions. Nuclei and mitochondria had much higher lead values than the other fractions. The microsomal and soluble protein fractions were least

affected. However, each fraction evidenced the pattern described.

Distribution of ^{210}Pb in Choroid Plexus, Brain, and Meninges of Normal and Lead-Poisoned Guinea Pigs: Modification by a Cardiac Glycoside.

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Previous studies showed a strong concentration of radiolabeled lead nitrate by choroid plexus (CP) in normal and lead-poisoned guinea pigs. The present study was designed to examine further the mechanisms of this uptake. In control animals, ^{210}Pb, 0.01 μCi/kg, was strongly concentrated at 5 min in CP and meninges [tissue-to-blood (T/B) ratios of 90, and 14] and poorly in brain (T/B = 0.32). By 240 min, T/B values had fallen to 1%, 27%, and 13% of 5-min values. The reduction in ^{210}Pb accumulation was identical with the decrease of total ^{210}Pb activity in CP, but less than the fall in brain and meningeal ^{210}Pb activity. Ouabain, $10^{-5}M$ (0.01 mg/kg), given intravenously, reduced 5 min accumulation of ^{210}Pb to 2% of CP control values and to 13% of control meningeal values but only by 30% in brain. In lead-poisoned animals, ouabain had no significant effect on tissue accumulation of ^{210}Pb. Thus CP ^{210}Pb levels reflect mainly exchange with blood, whereas brain and meningeal levels are controlled by additional factors, which may involve redistribution of CP lead. The ouabain effects strongly suggest that lead accumulation is associated with active cation transport in normal CP. These functions are altered in lead encephalopathy, and this may be an important factor in the pathogenesis of that disorder.

Low Level Lead Exposures: Behavioral Effects.

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In order to assess possible persisting behavioral changes in adulthood of early neonatal exposure to lead (Pb), Long-Evans hooded rat pups were daily treated with Pb acetate via a stomach tube from 1 to 30 days of age. Two or three pups per liter served as control animals, while the remaining five to seven pups in a given litter received one of the following doses of lead in a milk solution: 0.010, 0.025, 0.100, or 0.200 mg Pb/day/g body weight. These doses, respectively, produced the following blood Pb levels when tested in adulthood: 5.77, 6.85, 7.41, and <8.15 μg-% Pb. Subjects were tested for Pb effects on food and water consumption and body weight gain, general locomotor activity, aggressiveness toward mice, and learning in appetitively-motivated learning tasks, including a T-maze task and operant bar pressing for food reward on a DRL schedule of reinforcement. When tested in circular photocell activity cages at 40–45 days of age, male Pb-treated rats were markedly hyperactive, especially at the 0.025 dose level, while Pb-treated females were much less hyperactive. At 80–90 days of age, this sexual dichotomy was even more pronounced, with Pb-treated females at all dosage levels actually displaying slightly reduced activity levels. A dosage-dependent hyperactivity still persisted in male Pb-treated rats, i.e., rats in the 0.010 and 0.200 dosage groups did not differ from control levels, while the 0.025 group was clearly and markedly hyperactive and the 0.100 animals somewhat less so. In regard to the other behavioral measures observed, few differences could be detected between Pb and control rats. Pb rats from day 1 to 60 appear to eat slightly more than control subjects, probably secondary to the Pb hyperactivity, but no differences in consummatory behavior are apparent from 60 to 90 days. In addition, learning in either the T-maze or DRL task started after 90 days was not impaired by the neonatal Pb treatments. Also, aggressiveness to mice was largely unaffected, with the possible exception of reductions in muricide (mouse-killing) occurring at the 0.010 and 0.200 Pb levels. The most salient features of the present finding, then, are a demonstration of a sexual dichotomy in regard to lead-induced hyperactivity, with males displaying the syndrome much more markedly than females—analogous to the prevalence of hyperkinetic syndrome among male human children—a dosage-dependent relationship between hyperactivity and neonatal lead treatment, and the persisting nature of the hyperactivity into adulthood long after cessation of exposure to exgenous lead.

Toxicity of Metallic Chlorides and Oxides for Rabbit Alveolar Macrophages *in Vitro*.

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Cadmium, vanadium, nickel, manganese, and chromium are among those metals found at highest concentrations in the smallest particles collected from ambient air. In the intact animal, alveolar macrophages are exposed directly to soluble as well as in-

soluble forms of metallic air contaminants. Because of the central role of this cell type in pulmonary defense, experiments were performed to determine the relative cytotoxic effects of chlorides and oxides of the above named metals for the rabbit alveolar macrophage *in vitro*. Using a roller culture apparatus, attached cells in medium 199 were exposed for 20 hr at 37°C in a humidified atmosphere containing 4% CO_2 to concentrations of soluble metallic ions ranging from 0.01 to 10*mM*. Numbers of viable cells as compared to controls were reduced by 50% at the following concentrations: Cd^{2+}, 0.08*mM*; Vo_3^-, 0.10*mM*; Ni^{2+}, 3.8mM; Mn^{2+}, 4.7*mM*; Cr^{3+}, 5.0*mM*. Changes in cell viability (by exclusion of trypan blue) could be correlated with morphological alterations observed by scanning electron microscopy and with changes in specific activity of a lysosomal enzyme (acid phosphatase). Studies with soluble salts indicated that Cd^{+2} was unique in failing to promote cell lysis at concentrations that caused decrease in cell viability. Ni^{2+} was also unique in depressing phagocytic activity at concentrations lower than those affecting viability. Preliminary investigations with washed particles of metallic oxides indicated that most were intrinsically nontoxic or that toxicity could be related to solubility in the test system. The probable dependency of cytotoxicity upon particle size has not been investigated.

Interaction of Nickel Oxide and Influenza Infection in the Hamster. CURTIS D. PORT, JAMES D. FENTERS, and RICHARD EHRLICH, *III Research Institute, Chicago, Illinois,* and DAVID L. COFFIN and DONALD GARDNER, *Environmental Protection Agency, Research Triangle Park, North Carolina.*

The role of particulate dust in the development of respiratory disease has not been satisfactorily clarified, especially as related to infectious agents. These studies were conducted to determine the effects of 1, 2.5, and 5 mg of nickel oxide (NiO), <5 μM in size suspended in 0.5% gelatin-saline, on the susceptibility of hamsters to influenza infection. Male Syrian golden hamsters were challenged with influenza A/PR/8 virus followed by intratracheal injections of NiO within 1 hr. Mortality rates were not greater than those of the controls given influenza virus only, gelatin-saline only, or NiO. When the interval between virus challenge and exposure to particulate was increased to 24 hr, significantly increased death rates were observed in hamsters given all concentrations of NiO as well as gelatin–saline only. On the other hand, significantly increased mortality was noted only in hamsters given 5 mg of NiO 48 hr after infection. Death rates observed in the reverse NiO-infection sequence were markedly lower. Death did not occur upon exposure to only influenza virus, gelatin-saline, or NiO. Hamsters given NiO after infection showed mild to severe acute interstitial infiltrate of polymorphonuclear cells and macrophages 1 and 2 weeks later. Influenza-infected hamsters given gelatin–saline did not exhibit this type of interstitial reaction. Additional pathologic changes in the lungs included bronchial and bronchiolar epithelial hyperplasia, focal proliferative pleuritis, and adenomatosis. Thus, the data suggest that the additional presence of NiO can exacerbate an existing respiratory viral infection.